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DIFFERENTIAL RESPONSES OF FORAGE LEGUMES TO ALUMINUM

KEY WORDS: Alfalfa, birdsfoot trefoil, red clover, growth, nutrient uptake

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ABSTRACT

Response of alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and red clover (*Trifolium pratense* L.) to aluminum was evaluated in a nutrient culture system under controlled conditions. In each of the species, varietal differences were also compared. In the absence of Al stress, varieties of alfalfa and Tensas red clover produced more dry weight than the other legumes. However, among the legumes tested, alfalfa was the most sensitive to Al. Aluminum reduced the uptake of many of essential nutrients. Overall, red clover cultivars experienced the least reduction in elemental uptake, whereas alfalfa cultivars experienced the greatest reduction in uptake of elements under Al stress. The efficiency ratio (ER) assisted in differentiating legumes entries into efficient and inefficient utilizers of absorbed nutrients. The ER is defined as milligrams of dry shoot weight produced per milligram of element in the shoot. The presence of Al in the growth medium reduced the ER for all elements. With a few exceptions, ER for various elements, gave positive correlations with shoot weight. The

species and cultivars used in this study showed inter- and intraspecific differences in growth, uptake of nutrients and nutrient efficiency ratios in the presence or absence of Al stress.

INTRODUCTION

Aluminum toxicity is an important growth limiting factor in many acid soils and further, the problem is particularly serious in strongly acid subsoils that are difficult to lime (1,2). The majority of the soils of the Appalachian region are acidic and infertile and are incapable of supporting reasonable pasture production. Plants that can tolerate soil acidity have an advantage in achieving reasonable yields in these soils. Plant species and varieties within species are known to differ widely in their tolerance to aluminum (3,4). Varietal differences in tolerance to Al have been observed in many species of temperate and tropical legumes (5,6,7,8,9). Such differences have been attributed to modification of root rhizosphere environment, uptake, transport, and utilization of Al and essential nutrients (4). Further, it is evident that pasture legume species are more sensitive to soil acidity than pasture grass species (1,10). Genotypic and cultivar differences in shoot mineral concentrations have been reported in forage legumes (11,12,13,14). Differences in nutrient uptake and yield among cultivars of given species have been related to absorption, translocation, shoot demand, and dry matter production potentials per unit of nutrient absorbed (15,16,17). In the present study, three species of legumes, alfalfa, birdsfoot trefoil, and red clover were tested for their response to Al under climatically controlled conditions. Two cultivars were used for each of the species. The objectives were to evaluate shoot and root growth responses, mineral uptake, and mineral efficiency ratios in legume species subjected to various levels of Al in nutrient solutions.

MATERIALS AND METHODS

The experiment was conducted in a climatically controlled growth chamber with a light intensity of 530 $\mu E\ s^{-1} m^{-2}$, imposed for 14 hours daily. The relative humidity was maintained at 60%. Temperature of the growth room was 28°C in the light cycle and 22°C in the dark cycle.

Seedlings of birdsfoot trefoil (*Lotus corniculatus* L., varieties, 'Empire' and 'Viking'), red clover (*Trifolium pratense* L., varieties, 'Kenstar' and 'Tensas'), and alfalfa (*Medicago sativa* L., varieties, 'Arc' and 'Oklahoma') were raised in perlite medium under mist chambers. Nine day old seedlings were suspended into nutrient solution which was held in 14 liter polyethylene tubs. High density styrofoam was used as a top to hold plants in place. The nutrient solution used in the study was modified 1/5 strength Steinberg solution similar to the one proposed by Foy et al. (18). Three levels of Al (0, 100, 200 $\mu mol\ L^{-1}$ Al) were added as $Al_2(SO_4)_3 \cdot 18H_2O$. The Al was introduced into the solution 3 days after plants had been transplanted into the tubs. A complete randomized design with three replications was used. Solution pH was adjusted initially to 4.5 and left unadjusted thereafter. Water having a pH of 4.5 was added to the tubs to maintain the solution levels. During the growth, solution was continuously aerated. The experiment was terminated when plants were 51 days old. At harvest, shoot and root dry weight were recorded and plant samples were ground and digested in H_2O_2:H_2SO_4 mixture. Elemental determinations were made by inductively coupled plasma emission spectroscopy (ICP).

Relative growth reduction (RGR) for both shoot and roots, the efficiency ratio (ER) and percent inhibition of nutrient uptake (PI) were calculated as follows (11,16):

$$RGR = [1-(\text{Growth with Al}/\text{growth without Al})] \times 100 \quad [1]$$

$$ER = \text{Milligrams of dry shoot wt/milligram of element in shoot} \quad [2]$$

$$PI = [(U_0 - U_1)/U_0] \times 100 \quad [3]$$

where U_0 and U_1 refer to the total content of any given mineral element at 0 and 100 µmol L^{-1} Al, respectively.

The adopted legume species and their respective cultivars showed a greater degree of separation at 100 µmol L^{-1} Al, therefore this level of Al chosen for comparison of nutrient uptake, inhibition, and ER.

RESULTS AND DISCUSSION

Growth of Shoots and Roots

Plant growth of all species was markedly affected by Al (Table 1). In control treatment Tensas red clover and both varieties of alfalfa produced twice as much dry matter as did other legume entries. At 100 µmol L^{-1} Al, both cultivars of red clover outperformed the other entries. At this level of Al both cultivars of alfalfa and Empire birdsfoot trefoil recorded more than 90% reduction in shoot weight compared with the control treatment. At 200 µmol L^{-1} Al growth in all the species was reduced by more than 97% compared with the control.

Growth of shoots and roots showed significant differences with respect to Al levels. With the exception of species X Al interaction for root wt; species, varieties and their interaction with treatment aluminum (T-Al) were nonsignificant for shoot and root parameters. Treatment Al effects were subdivided into linear and quadratic components to investigate the various responses of shoot and root weights and RGR to the Al treatments. Significant linear and quadratic responses to the T-Al for growth and RGR of shoots and roots were observed. Root

TABLE 1

Growth and Relative Reduction of Growth (RGR) for Shoot and Root of Legumes at Various Al Levels.

Species/ Varieties	Growth 0 µmol L^{-1} Al Shoot	Root	RGR 100 µmol L^{-1} Al Shoot	Root	200 µmol L^{-1} Al Shoot	Root
	--g/10 plants--		--------------%		---------------	
Alfalfa						
Arc	6.40	3.05	98	97	99	99
Oklahoma	6.22	2.59	99	98	99	98
Birdsfoot Trefoil						
Empire	3.50	2.42	90	88	99	99
Viking	3.53	2.68	87	87	98	99
Red clover						
Kenstar	3.85	1.80	71	78	97	98
Tensas	6.07	1.63	64	63	98	97

Analysis of Variance - F Value

Source of Variance		Shoot Wt.	Root Wt.	RGR-Shoot	RGR-Root
Species	(S)	2.6NS	1.2NS	0.86NS	0.8NS
Varieties	(V)	1.0NS	0.2NS	0.01NS	0.1NS
Treatment-Al	(T-Al)	69.3**	118.3**	76.70**	106.1**
S X T-Al		3.1NS	4.0**	0.75NS	0.7NS
V X T-Al		0.4NS	0.2NS	0.02NS	0.1NS
Among-T-Al					
Linear		117.4**	196.6**	130.45**	179.8**
Quadratic		21.2**	39.9**	22.95**	32.4**

*, **Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant.

weight of all the entries were significantly correlated with their respective shoot weight (Table 2). From the present results, legumes used in this study apparently are more Al sensitive than certain other crops (2, 4, 23). Aluminum levels less than 100 µmol L^{-1} might have given better separation of the legumes used in this study.

Uptake and Inhibition of Nutrients

Since growth of all the legumes was poor at 200 µmol L^{-1} Al, comparisons of nutrient uptake and percent nutrient inhibition were made at the 100 µmol L^{-1} treatment only (Table 3).

TABLE 2
Correlation Coefficient (r) Relating Shoot Weight of Alfalfa, Birdsfoot Trefoil, and Red Clover to Root Growth, Nutrient Uptake, and ER.

Variables	Alfalfa	Birdsfoot Trefoil	Red Clover
Root wt.	0.97**	0.99**	0.89**
RGR-Root	-0.97**	-0.99**	-0.90**
RGR-Shoot	-0.99**	-0.99**	-0.96**
U-P	0.99**	0.98**	0.99**
U-K	0.99**	0.98**	0.71**
U-Mg	0.99**	0.97**	0.98**
U-Cu	0.99**	0.97**	0.91**
U-Fe	0.98**	0.60**	0.88**
U-Mn	0.98**	0.93**	0.99**
U-Zn	0.99**	0.70**	0.99**
ER-P	0.91**	-0.34NS	-0.35NS
ER-K	0.95**	0.92**	0.77**
ER-Mg	0.80**	0.42NS	0.58*
ER-Cu	0.89**	0.65**	-0.19NS
ER-Fe	0.90**	-0.57*	-0.65**
ER-Mn	0.61**	0.28NS	0.73**
ER-Zn	0.87**	0.53*	0.74**

*, **Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant.

Tensas red clover was the most efficient and Oklahoma alfalfa was the most inefficient in uptake of nutrients. This reflects higher or lower shoot dry weight in these cultivars. Aluminum at 100 µmol L^{-1} reduced shoot dry weight of Tensas red clover by 64% and that Oklahoma alafalfa by 94%.

Overall, the uptake of nutrients showed a highly positive correlation with shoot wt. of all the legume species (Table 2). Plant demand for any given nutrient is a function of its internal ionic concentrations and rate of growth (19,20,21). Aluminum is known to interfere with the uptake, transport, and use of several essential elements (4,15). The reduction in growth due to Al in the growth medium created a lower internal demand and in turn,

TABLE 3

Nutrient Uptake (U) and Percent Inhibition of Uptake (PI) at 100 µmol L^{-1} Al in Different Legume Species/Cultivars.

Element	Alfalfa		Birdsfoot Trefoil		Red clover	
	Arc	Oklahoma	Empire	Viking	Kenstar	Tensas
			Uptake (U)†			
P	0.06	0.04	0.25	0.22	0.75	1.55
K	1.00	0.69	3.48	3.76	14.73	26.39
Mg	0.22	0.15	0.61	1.01	2.19	3.20
Cu	1.19	1.00	1.46	1.83	5.26	7.44
Fe	3.50	2.69	11.46	15.12	28.94	49.44
Mn	4.66	4.02	10.52	16.40	34.75	55.89
Zn	6.53	4.45	9.81	11.79	30.17	47.14
			Percent Inhibition (PI)			
P	98	98	86	87	61	65
K	95	97	72	70	-29	-20
Mg	95	96	84	78	62	72
Cu	94	93	81	81	96	98
Fe	96	96	88	94	85	82
Mn	97	97	87	86	63	64
Zn	94	94	79	82	51	63

†U = mg•10 $plants^{-1}$ for P, K, and Mg; µgram•10 $plants^{-1}$ for Cu, Fe, Mn and Zn.

this lowered the total uptake. In our study, 100 µmol L^{-1} Al reduced nutrient uptake by 61 to 98% relative to the control (Table 3). However, in red clover cultivars, K uptake was increased in the presence of the 100 µmol L^{-1} Al. Overall, red clover cultivars experienced the least reduction in elemental uptake by Al; whereas, alfalfa cultivars experienced the greatest reduction in uptake of all the elements under consideration.

Significant differences in uptake of nutrients were noted only for Al treatments (Table 4). Significant effects for species were noted for uptake of P, K, Mg, and Cu; however,

TABLE 4

Analysis of Variance (F value) for Treatment and Interaction Effects on Nutrient Uptake and Efficiency Ratios in Various Legume Species.

Source of Variation	P	K	Mg	Cu	Fe	Mn	Zn
				Uptake			
Species (S)	6.5**	7.5**	8.7**	18.3**	0.9NS	1.2NS	0.3NS
Varieties(V)	2.6NS	1.5NS	1.2NS	2.1NS	0.9NS	0.4NS	0.9NS
Treat-Al (T-Al)	57.9**	31.5**	54.4**	23.9**	5.5**	43.5**	16.4**
S X T-Al	1.9NS	6.6**	2.5NS	16.8**	0.8NS	1.9NS	0.3NS
V X T-Al	1.1NS	0.5NS	0.6NS	2.0NS	0.9NS	0.2NS	0.8NS
T-Al							
Linear	101.6**	62.9**	96.0**	36.7**	8.7**	74.0**	28.0**
Quadratic	14.2**	0.1NS	12.7**	11.2**	2.2NS	13.1**	4.8*
				ER			
Species (S)	57.8**	13.6**	15.6**	33.4**	2.9NS	7.5**	1.4NS
Varieties (V)	5.9**	0.7NS	0.4NS	0.7NS	12.8**	1.0NS	0.9NS
Treat-Al (T-Al)	74.1**	862.4**	55.0**	73.6**	21.9**	18.1**	122.9**
S X T-Al	57.6**	4.2**	27.3**	51.7**	130.4**	5.5**	9.3**
V X T-Al	1.4NS	3.8**	0.9NS	0.7NS	4.4**	1.0NS	1.9NS
T-Al							
Linear	38.0**	1192.9**	79.6**	144.3**	7.3*	31.4**	239.2**
Quadratic	110.3**	531.9**	30.6**	2.9NS	36.5**	4.7*	6.7*

*,**Significant at 0.05 and 0.01 levels of probability, respectively.
NS = Not significant.

varieties gave nonsignificant effects with uptake of all the elements. Significant interaction effects for species and T-Al were noted only for K and Cu. Aluminum treatment versus uptake for all elements had highly significant linear components; whereas, uptake of P, Mg, Cu, Mn, and Zn had significant quadratic components.

Efficiency Ratios (ER)

The ER assists in differentiating legume entries into efficient and inefficient utilizers of the absorbed nutrients. Such an evaluation of species and cultivars within species

TABLE 5

Efficiency Ratios (ER) for Nutrients in Shoots of Legumes Species/Cultivars at 0 and 100 $\mu mol\ L^{-1}$ Al Treatments.

Element	Alfalfa		Birdsfoot Trefoil		Red clover	
	Arc	Oklahoma	Empire	Viking	Kenstar	Tensus
			0 $\mu mol\ L^{-1}$ Al			
P	2522	2581E†	1838	2061	1711	1357I
K	308E	279	260I	290	275	286
Mg	1463E	1367	827	837	576	558I
Cu	312	360	400E	379	26	20I
Fe	70	80E	33	19	16I	21
Mn	43E	39	39	34I	34I	42
Zn	65	73E	69	47I	53	51
			100 $\mu mol\ L^{-1}$ Al			
P	1554	1618	1299I	1860E	1441	1386
K	99	100	103	111E	78I	86
Mg	458	467	589	223I	515	665E
Cu	83	69I	221	223	206	280E
Fe	28	26I	29	29	38	42E
Mn	21	17I	33	26	32	39E
Zn	15I	16	33	37	36	47E

†E = Most Efficient; I = Most Inefficient. ER for Cu, Fe, Mn, and Zn need to be multiplied by 10^3.

appears to be especially important under Al stress. Plants that have a high ER under Al stress might be able to perform well in infertile acidic soils. In alfalfa, the ER for all elements showed a positive relationship with shoot weight (Table 2). In birdsfoot trefoil, with the exceptions of ER for P and Fe, the ER for other elements showed a positive relationship with shoot weight. In red clover, shoot weight was positively related to the ER of K, Mg, Mn and Zn.

The ER for various elements in legume species at 0 and 100 $\mu mol\ L^{-1}$ Al are shown in Table 5. With the exception of Cu in Red clover cultivars, the presence of Al in growth medium reduced the magnitude of ER for all the elements under consideration. In the absence of Al, cultivars of alfalfa in general gave the highest ER values for P, K, Mg, Fe, Mn and Zn and while cultivars of Red clover generally had the lowest ER values. Arc alfalfa was the most efficient utilizer of absorbed K, Mg and Mn; whereas, Oklahoma alfalfa was the most efficient utilizer of P, Fe, and Zn. In the absence of Al Tensas red clover was the most inefficient utilizer of absorbed P, Mg and Cu; whereas, Kenstar red clover was the most inefficient utilizer of absorbed Fe and Mn. At 100 $\mu mol\ L^{-1}$ Al, red clover cultivars in general gave the highest ER values for Mg, Cu, Fe, Mn and Zn. Tensas red clover was the most efficient in utilization of absorbed Mg, Cu, Fe, Mn and Zn; whereas, Viking birdsfoot trefoil was the most efficient utilizer of P and K

The inter- and intraspecific differences in mineral uptake and utilization in various crops is well documented (11,15,16,17, 22,23). With a few exceptions, T-Al, species and cultivars, and their interactions significantly affected the efficiency ratios (Table 4). A significant linear and quadratic response to Al was observed for the ER values of different nutrients.

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